Guidance for Industry

Safety Testing of Drug Metabolites

DRAFT GUIDANCE

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Center for Drug Evaluation and Research (CDER)

June 2005
Pharmacology and Toxicology

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I. INTRODUCTION

This guidance makes recommendations on when and how to identify, characterize, and evaluate the safety of unique human metabolites and major metabolites of small molecule (nonbiologic) drug products. These metabolites may not be adequately assessed during standard nonclinical studies because they occur only in humans (unique metabolite), or at much higher levels (major metabolite) in humans than in the species used during standard nonclinical toxicology testing. If such metabolites are identified, they should be evaluated as early as possible during the clinical development program. This guidance defines *major metabolites* primarily as those identified in human plasma that account for greater than 10 percent of drug related material (administered dose or systemic exposure whichever is less) and that were not present at sufficient levels to permit adequate evaluation during standard nonclinical animal studies.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

Traditionally, drug metabolites in general have not been routinely evaluated in cross-species safety assessments because their specific contribution to the overall toxicological potential of the parent drug has been unknown. With the availability during the past

[1] This guidance has been prepared by the Pharmacology and Toxicology Coordinating Committee (PTCC) in the Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration.

decade of technologies that can identify, measure, and characterize metabolites, we have gained a better understanding of the role metabolites play in drug safety assessment.

Generally, we have used measurements of circulating concentrations of a parent drug in animals as an index of systemic exposure in humans. Quantitative and qualitative differences in metabolite profiles are important when comparing exposure and safety of a drug in a nonclinical species relative to humans during risk assessment. Based on data obtained from in vitro and in vivo metabolism studies, when the metabolic profile of a parent drug is similar qualitatively and quantitatively across species, we can generally assume that potential clinical risks of the parent drug and its metabolites have been adequately characterized during standard nonclinical safety evaluations. However, metabolic profiles and metabolite concentrations can vary across species, and there are cases when clinically relevant metabolites have not been identified or adequately evaluated during nonclinical safety studies. This may be because the metabolite being formed in humans was absent in the animal test species (unique human metabolite) or because the metabolite was present at much higher levels in humans (major metabolite) than in the species used during standard toxicity testing.

The Agency recommends that — and this guidance encourages — attempts be made to identify as early as possible during the drug development process differences in drug metabolism in animals used in nonclinical safety assessments compared to humans (Baillie and Cayen et al. 2002; Hastings et al. 2003). It is especially important to identify metabolites that may be unique to humans. The discovery of unique or major human metabolites late in drug development can cause development delays and could have possible implications for marketing approval. Early identification of unique or major metabolites will allow for timely assessment of potential safety issues.

Generally, we recommend that metabolites identified in human plasma that account for greater than 10 percent of drug related material (administered dose or systemic exposure whichever is less) be considered for safety assessment. The rationale for setting the level at greater than 10 percent for characterization of metabolites reflects consistency with other FDA and EPA regulatory guidances (U.S. Food and Drug Administration 2002; U.S. Environmental Protection Agency 1998) and is supported by actual cases, described below, in which it has been determined that the toxicity of a drug could be attributed to one or more metabolites present at greater than 10 percent of the administered dose. Of the cases that follow, the last two are examples of a situation when a metabolite present at less than 10 percent caused toxicity. As a result, depending on the situation, some metabolites present at less than 10 percent should also be tested.

- Halothane, an inhalation anesthetic, has a metabolite, trifluoroacetylchloride, which represents less than 20 percent of the administered dose. Yet this metabolite is responsible for halothane-induced liver toxicity, a major safety concern that has led to limited use of the drug (Pohl et al. 1989).

- Use of felbamate for the treatment of several forms of epilepsy has been associated with adverse events of aplastic anemia and hepatotoxicity that are

attributed to a reactive metabolite, atropaldehyde, which was detected indirectly as the urinary metabolites mercapturic acid (2.3 percent of felbamate concentration in urine) and mercapturic alcohol (13.4 percent of felbamate concentration in urine) (Thompson et al. 1999).

- The anticancer drug, cyclophosphamide, has no direct cytotoxic action. However, its toxicity is attributed to a number of metabolites. One of these metabolites, 4-hydroxycyclophosphamide, represented approximately 8.3 percent of the total plasma exposure (Sladek et al. 1984).

- Acetaminophen liver toxicity is attributed to N-acetyl-p-benzoquinone imine (NAPQI), a toxic reactive intermediate of acetaminophen, detected in urine as thioether metabolites. The latter were found to constitute approximately 9 percent of a therapeutic dose of acetaminophen (Manyike et al. 2000).

III. SAFETY TESTING AND NONCLINICAL STUDY DESIGN

Drugs entering the body undergo biotransformation via Phase I and Phase II metabolic pathways. Based on the nature of the chemical reactions involved, metabolites formed from Phase I reactions (e.g., oxidation, reduction) are more likely to be pharmacologically active, and require safety evaluation, than Phase II products (e.g., glucuronidation, sulfation). Although conjugated metabolites from Phase II reactions are generally pharmacologically inactive, more water soluble, and readily eliminated from the body, some are toxic. Sulfate and some glucuronide metabolites (e.g., acyl glucuronides of carboxylic acids) may retain pharmacological activity as well as toxicity of the parent drug and may require toxicological evaluation. Demonstration that a metabolite is pharmacologically inactive at the target receptor does not guarantee that it is not toxic, however. If the unique or major metabolites are suspected to contain a reactive functional group, it is important to assess the toxicity potential of these reactive metabolites. Chemically reactive intermediates are rarely detectable due to their short half-life, although stable products (i.e., glutathione conjugates) resulting from such intermediates can provide some indication of exposure to these potentially toxic species.

Generally, compounds with the following characteristics are of particular concern and may warrant additional investigation:

- Narrow therapeutic indices
- Significant toxicity
- Significantly diverse metabolic profiles between human and nonclinical species
- Irreversible toxicity, or adverse effects not readily monitored in the clinic

A. Goals of Safety Testing

The objectives of standard nonclinical safety studies are to evaluate the general toxicity profile of a drug and its metabolites in rodent and nonrodent animal species and to assess

the potential for genotoxicity in support of phase 1 safety and tolerability studies in humans. Metabolism studies are generally performed through a combination of in vitro studies using animal and human tissues and in vivo studies in animals. The in vitro studies are generally conducted prior to the in vivo studies and provide an initial comparative metabolic profile. Results from these studies can assist in the selection of the appropriate animal species for toxicological assessments, should qualitative interspecies differences in metabolism be detected.

Identifying a major metabolite in animals that does not exist in humans can mean that a toxicity observed in that animal species may not be relevant to humans. Conversely, identifying a human metabolite during clinical development that did not form at appreciable levels in animals would raise safety concerns because it probably was not evaluated in the nonclinical studies due to inadequate exposure. Additionally, when a potentially clinically relevant toxicity is observed during standard nonclinical studies, it is prudent to determine if metabolites contribute to that finding. In such cases, we recommend that the metabolites be synthesized and directly administered to the appropriate animal species for further pharmacological/toxicological evaluation. When qualitative and/or quantitative species differences in metabolite profiles are discovered, we also recommend investigation of different routes of administration or use of alternative animal species for safety assessments.[2]

B. Identification of Metabolites

In vitro studies using liver slices, microsomes, or hepatocytes from animals and humans to identify the drug metabolic profile are generally conducted before initiation of clinical trials. It is important to also try to determine whether the concomitant use of drugs results in the inhibition or the induction of common metabolic pathways. In vivo metabolic profiles in nonclinical test species are generally available early in drug development, and their results may reveal significant quantitative and/or qualitative differences in metabolism across species. However, a unique metabolite may only be recognized after completion of in vivo metabolic profiling in humans. Therefore, we recommend the in vivo metabolic evaluation in humans be performed as early as feasible.

In general, systemic exposure to metabolites varies among species, and it is uncommon for humans to form unique metabolites. Therefore, identification of major human metabolites at levels higher than those measured in the test species used for toxicological assessment is of serious concern. For metabolites detected in humans as well as in nonclinical species (although at lower levels in the latter), adequacy of exposure should be considered on a case-by-case basis. Generally, systemic exposure is assessed by measuring the concentration of the compound in serum or plasma. However, when measurements cannot be made in plasma for any one or a number of reasons, measurements can be made in other biological matrices such as urine, feces, or bile. Noncirculating metabolites (i.e., excreted in bile, urine) are sometimes identified before clinical trials, but are not usually monitored. It is quite likely that excreted metabolite

[2] Discovery of such a metabolite could delay development until the relationship between metabolite exposure and toxicity is understood.

levels may be a more appropriate metric in many instances. For example, if Phase II conjugation products of a metabolite are present in the excreta, it can be assumed that systemic exposure to the metabolite has occurred. We recommend consulting the ICH Q3A guidance[3] with regard to the development of analytical methods for measuring metabolites in selected matrices. If the systemic exposure in nonclinical species is equivalent to human exposure when measured in plasma and/or excreta, levels may be considered sufficient and alleviate the need for additional toxicity testing. We encourage contacting the Agency early in drug development to discuss these issues.

C. Structure Activity Relationships

Technical advancement has led to the development of analytic instrumentation (e.g., GC/MS, LC/MS, MS/MS) with improved sensitivity. It is now possible to identify the molecular structures of metabolites early in drug development. With the availability of computational software designed to predict activity relative to a known structure, the mutagenic, carcinogenic, or teratogenic potential of a drug or a metabolite can be evaluated as soon as a structure is identified. Although structure activity relationship analyses are not considered a substitute for actual testing, we encourage submission of the results from these analyses.

D. General Considerations for Nonclinical Study Design

When designing a nonclinical study for a unique or major metabolite, it is important to consider physicochemical characteristics of the metabolite, including solubility, permeability, extent of absorption, route of administration, and exposure. The indicated patient population, duration of use, and exposures at the therapeutic dose are also important considerations for the risk assessment. Another important consideration is the potential for biotransformation of directly administered human metabolites in animals as well as the presence of impurities in the synthesized metabolites.

It is important to consider combined exposure to parent and pharmacologically active metabolites in safety assessments. A pharmacologically active metabolite can be more, equal, or less active than the parent drug at the target receptor. Similarly, a metabolite may cause toxicity by (1) eliciting exaggerated pharmacological effects via the target receptor, (2) activating receptors different from the parent drug target receptors, or (3) through nonreceptor mediated mechanisms (e.g., physico-chemical).

[3] ICH guidance for industry *Q3A Impurities in New Drug Substances.*

IV. RECOMMENDED STUDIES FOR ASSESSING THE SAFETY OF METABOLITES[4]

Four kinds of safety studies can be performed to assess the safety of a unique or major metabolite. They are described briefly here.

A. General Toxicity Studies

The potential toxicity of a unique or major human metabolite should be evaluated to enable comparisons between the metabolite and its parent compound. A general toxicity study with direct dosing of the metabolite can range from a minimum duration of 14 days to a maximum duration of 90 days (ICH Q3B(R)).[5] It is important to justify the duration and dose of the study based on available relevant information and clinical use. For metabolites that are found to be more toxic than the parent compound and/or that have a different toxicity profile, such as delayed toxicity, different target organs, or toxicity not readily monitorable with available biomarkers, toxicology studies of longer duration (i.e., 6 months for rodents, 9 months for nonrodents) may be warranted on a case-by-case basis. An important objective is to identify dose-dependent toxicity. We recommend that the maximum dose either elicit frank toxicity without causing excessive incidence of morbidity/death or be the maximum feasible dose up to 2000 mg/kg/day. We recommend performing the study in the appropriate animal species most likely to maximize the potential to detect the toxicity of a metabolite. We also recommend using the intended clinical route of administration of the product; however, other routes (e.g., intravenous, intraperitoneal) may be used to achieve sufficient exposure. It is crucial to gather toxicokinetics data from this study to ensure adequate exposure. On a case-by-case basis, an ECG evaluation may help assess the potential for QT prolongation (ICH S7B).[6] Mechanistic studies to assess specific toxicity endpoints may also be warranted based on the results of the general toxicity studies.

B. Genotoxicity Studies

We recommend that the potential genotoxicity of the metabolite be assessed in a minimal genotoxicity screen that consists of one in vitro assay to detect point mutations and another to detect chromosomal aberrations. It is important that these assays be conducted according to recommendations in the guidances ICH S2A and S2B.[7] If one or both of the

[4] See Appendix A: Decision Tree Flow Diagram. This diagram describes when and which studies are needed to determine safety of the drug metabolite.

[5] ICH guidance for industry *Q3B(R) Impurities in New Drug Products.*

[6] ICH guideline for industry *S7B The Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals*.

[7] ICH guidance for industry *S2A Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals* and ICH guidance for industry *S2B Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals*.

in vitro tests are equivocal and/or positive, performance of the complete standard battery of genotoxicity studies may be warranted.

C. Embryo-Fetal Development Studies

When a drug is intended for use in a population that includes women of childbearing potential, we recommend that an embryo-fetal developmental toxicity study be performed. The Agency may request other reproductive toxicity studies on a case-by-case basis depending on results of the general toxicity and embryo-fetal developmental studies. We recommend that reproductive toxicity studies be conducted in accordance with the guidances ICH S5A and S5B.[8]

D. Carcinogenicity Studies

The FDA may request carcinogenicity studies on a case-by-case basis for metabolites of drugs that are administered continuously for at least 6 months, or for metabolites of drugs used intermittently in the treatment of chronic or recurrent conditions.

Factors that might lead to such a request include existence of positive genotoxicity findings, genotoxic or carcinogenic structural alerts, tissue proliferative effects (i.e., hyperplasia, preneoplastic lesions) identified in general toxicology studies as well as any other relevant data. We recommend performing a single, 2-year rodent bioassay, but addition of a metabolite dose group to the oncogenicity study for the parent drug may be considered for nongenotoxic metabolites. Guidances ICH S1A, S1B, S1C, and S1C(R)[9] contain recommendations on carcinogenicity studies.

V. TIMING OF SAFETY ASSESSMENTS

Early identification of unique human or major metabolites can provide clear justification for nonclinical testing in animals, assist in planning and interpreting clinical studies, and prevent delays in drug development. Sponsors are encouraged to conduct in vitro studies to identify and characterize unique human or major metabolites early in drug development. If toxicity studies of a human metabolite are warranted, we recommend studies be completed and the study reports be submitted to the Agency before beginning large-scale phase 3 trials. In some cases, it may be appropriate for these nonclinical safety studies with unique human metabolites to be conducted before phase 3 studies; for example, (1) if the metabolite belongs to a chemical class with known toxicity; (2) if the

[8] ICH guidance for industry *S5A Detection of Toxicity to Reproduction for Medicinal Products* and ICH guidance for industry *S5B Detection of Toxicity to Reproduction for Medicinal Products: Addendum on Toxicity to Male Fertility*.

[9] ICH guidance for industry *S1A The Need for Long-Term Rodent Carcinogenicity Studies of Pharmaceuticals*, ICH guidance for industry *S1B Testing for Carcinogenicity of Pharmaceuticals*, ICH guidance for industry *S1C Dose Selection for Carcinogenicity Studies of Pharmaceuticals*, and ICH guidance for industry *S1C(R) Guidance on Dose Selection for Carcinogenicity Studies of Pharmaceuticals: Addendum on a Limit Dose and Related Notes.*

metabolite has positive structural alerts for genotoxicity, carcinogenicity, or reproductive toxicity; or (3) if clinical findings suggest the metabolite or related compounds have indicated special clinical safety concerns, such as QT prolongation.

To optimize and expedite development of drugs for serious or life-threatening diseases that lack an approved effective therapy, the number of nonclinical studies for the unique or major human metabolites may be limited on a case-by-case basis. We recommend sponsors contact the relevant review division to discuss such situations.

GLOSSARY

Major metabolite — A metabolite in humans that accounts for plasma levels greater than 10 percent of the administered dose or systemic exposure, whichever is less.

Metabolite — A compound derived from the parent compound through Phase I and/or Phase II metabolic pathways.

Pharmacologically active metabolite — A metabolite that has pharmacological activity at the target receptor that is greater than, equal to, or less than the parent compound.

Unique human metabolite — A metabolite produced only in humans.

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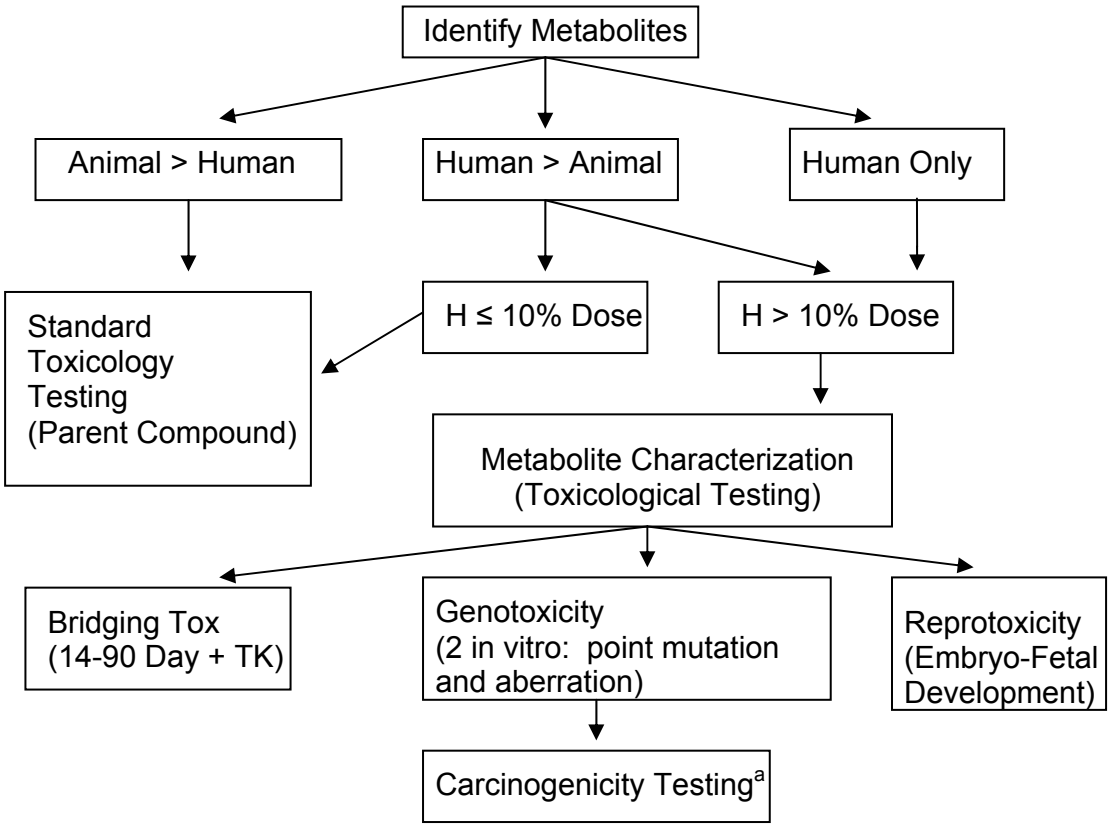

[a] Carcinogenicity testing may be needed on a case-by-case basis, independent of the results of genotoxicity testing (see Section IV.D).

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